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Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis

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Abstract: BACKGROUND AND PURPOSE: Optical coherence tomography (OCT) has shown thinning of the retinal nerve fibre layer (RNFL) and total macular volume (TMV) in multiple sclerosis (MS) patients. Measures of retinal atrophy are associated with the brain parenchymal fraction (BPF) assessed by magnetic resonance imaging (MRI). However, in MS, data on the relation of OCT measures and grey and white matter volumes are contradictory. We performed a prospective cross-sectional study with a statistically pre-defined endpoint to test our hypothesis that OCT measures of neuro-axonal degeneration are related to global and partial brain atrophy in early forms of MS. METHODS AND RESULTS: Forty-four patients with clinically isolated syndrome (n = 10) or relapsing-remitting MS (n = 34; mean disease duration = 3.2 years, median EDSS = 1.5) were enrolled in the study. Peripapillary- and volumetric OCT scans of the macula were performed using latest spectral-domain OCT technology. BPF as well as white and grey matter fractions (WMF/GMF) were assessed by 1.5 Tesla MRI scans. Generalized estimating equation models adjusted for age and linear regression statistics were used to assess the association between OCT and MRI measures. RNFL thickness, TMV and age were significantly associated with BPF. RNFL thickness and TMV independently predicted WMF ($P = 0.003$ and $P = 0.032$) but not GMF ($P = 0.717$ and $P = 0.357$) when corrected for age. In contrast, age was strongly associated with GMF ($P < 0.001$) but not WMF. CONCLUSION: Our study suggests that, in early MS, OCT measures of retinal atrophy are related to volumetric changes in the white but not grey matter compartment as assessed by MRI. It further substantiates the association of retinal thinning and brain tissue loss in MS.

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Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis

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Summary

Optical coherence tomography (OCT) has shown thinning of the retinal nerve fibre layer (RNFL) and total macular volume (TMV) in multiple sclerosis (MS) patients. Measures of retinal atrophy are associated with the brain parenchymal fraction (BPF) assessed by MRI. However, in MS, data on the relation of OCT measures and grey and white matter volumes are contradictory.

We performed a cross-sectional study with a statistically pre-defined endpoint to test our hypothesis that OCT measures of neuro-axonal degeneration are related to global and partial brain atrophy in early forms of MS.

44 patients with clinically isolated syndrome (CIS, n=10) or relapsing remitting MS (RRMS, n=34) (mean disease duration=3.2 years, median EDSS=1.5) were enrolled in the study. Peripapillary- and volumetric OCT scans of the macula were performed using latest spectral-domain OCT technology. BPF as well as white and grey matter fractions (WMF/GMF) were assessed by 1.5 T MRI scans. Generalized estimating equation models (GEE) adjusted for age and linear regression statistics were used to assess the association between OCT and MRI measures.

RNFL thickness, TMV and age were significantly associated with BPF. RNFL thickness, and TMV independently predicted WMF ($p=0.003$ and $p=0.032$) but not GMF ($p=0.717$ and $p=0.357$) when corrected for age. In contrast, age was strongly associated with GMF ($p<0.001$) but not WMF.

Our study suggests that, in early MS, OCT measures of retinal atrophy indicate white- but not grey matter damage as assessed by MRI. It further substantiates the association of retinal thinning and brain tissue loss in MS.

Keywords: Multiple Sclerosis, Optical Coherence Tomography (OCT), Retinal Nerve Fibre Layer, Brain parenchymal fraction, Atrophy

Abbreviations: OCT = optical coherence tomography; RNFL = retinal nerve fibre layer; TMV = total macular volume; BPF = brain parenchymal fraction; WMF = white matter fraction; GMF = grey matter fraction; TIV = total intracranial volume; BHFr = black hole fraction

Introduction

Demyelination caused by T cell-induced autoimmune inflammation has long been considered the primary hallmark of multiple sclerosis (MS). There is, however, increasing evidence that axonal damage and neuronal loss contribute significantly to irreversible functional deficits in MS [1]. Axonal damage occurs within inflammatory lesions [2-4] but also remote from sites of autoimmune inflammation [5,6]. Of note, axonal degeneration starts very early in the disease course and has been found in both active and inactive plaques [4].

Magnetic resonance imaging (MRI) is the best established structural measure to capture disease activity in MS. Conventional MRI sequences are sensitive for the monitoring of inflammatory MS lesions. In addition, the quantification of T2 lesions at the time of diagnosis has limited predictive value with respect to future disability evolution [7]. However, the association of MRI markers of inflammation and disability progression throughout the further disease course is weak [8,9]. In contrast, MRI measures of degeneration and atrophy appear to correlate better with disability evolution. The brain parenchymal fraction (BPF) or, conversely, ventricular enlargement, are widely used MRI outcomes to depict global brain atrophy in MS [9,10]. An inverse relation between BPF and disability progression has repeatedly been demonstrated [11,12]. Further, MRI-based stratification by the amount of inflammatory lesions and the extent of both global and focal brain atrophy during consecutive, monthly MRI scans has been used to dissect disease heterogeneity in MS [13]. Interestingly, disease progression over time was most pronounced in the subgroup with both high inflammation and degenerative changes [13]. Assessing atrophy parameters employs three-dimensional MRI sequences and segmentation

algorithms, that are, however, time consuming and not easily accessible in routine clinical practice. Furthermore, a number of confounding factors like inflammatory edema, hydration status, anti-inflammatory treatments and age have been shown to impact on the results of such atrophy measures by MRI [14, 15].

Optical coherence tomography (OCT) studies have consistently shown thinning of the retinal nerve fibre layer (RNFL) and total macular volume (TMV) in MS, both in patients with and without a history of acute optic neuritis (ON) [16]. Based on these data, the RNFL thickness (RNFLT) and TMV have been proposed as potential surrogate outcome measures of neuro-axonal degeneration in MS [14]. Recent studies suggest that OCT measures of retinal degeneration correlate with MRI measures of brain tissue loss [17-21]. As a limitation of all these studies, time domain OCT (TD-OCT) has been used as opposed to the more advanced and more accurate spectral domain OCT technique (SD-OCT), with faster data acquisition, greater reliability and higher resolution [17, 22]. Moreover, these previous studies were either carried out in small- [20, 23], or heterogeneous groups of patients [17, 18] or rather late in the disease course [17, 23].

There is little doubt that neurodegenerative processes become more and more prominent during more advanced disease stages like secondary progressive MS (SPMS). However, as axonal degeneration is already detectable as early as at the primary manifestation of the disease, the so-called clinically isolated syndrome (CIS), there is a need to investigate the relationship between OCT measures of retinal axonal degeneration and MRI measures of brain tissue loss early in the disease course [4].

We therefore designed a highly powered (95%), cross-sectional study with a statistically pre-defined endpoint applying latest OCT technology (spectral domain

Spectralis® OCT) to test the hypothesis, that OCT measures of neuronal and axonal degeneration are related to MRI measures of global brain atrophy (BPF) and regional volumes (grey and white matter fraction) already at very early disease stages.

Patients and Methods

Patients

Forty-four patients with clinically isolated syndrome suggestive of MS (CIS (n=10)) or an established diagnosis of RRMS (n=34) according to the 2005 revised McDonald criteria [24] were prospectively enrolled at the MS Outpatient- and Day Clinic at the Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (inims), University Medical Center Hamburg Eppendorf, Hamburg, Germany. All patients met the following criteria: age between 18 and 60 years, disease duration (as defined by time since first reported symptoms) not exceeding ten years, no acute relapse and no systemic steroid treatment within 30 days prior to study entry. Patients had to be off immunomodulatory treatment with interferons for at least 90 days before enrolment. Treatment with the monoclonal antibody natalizumab or glatirameracetate had to be stopped 6 months before study entry. Patients were scanned twice by MRI, at baseline and after an interval of six to twelve months. Patients with a CIS were only included if they showed one or more lesions in at least two out of four anatomical regions defined by the Barkhof criteria for dissemination in space. Neurological disability was assessed by a trained neurologist, blinded for MRI and OCT results, using the Expanded Disability Status Scale (EDSS) [25] and the Multiple Sclerosis Functional Composite (MSFC) [26]. Visual acuity was determined

using Snellen charts. Patients did not suffer from any metabolic, ophthalmological or neurological disorder other than MS by history. Patients with severe myopia (refraction error ≥ 6.0) were excluded from the study. Written informed consent was obtained from all patients. The study was approved by the Ethical Committee of the Board of Physicians in the State of Hamburg.

Methods

Magnetic Resonance Imaging

Brain MRIs were performed on a 1.5 Tesla scanner (Magnetom Sonata, Siemens, Erlangen, Germany) with a standard head coil. It consisted of a multiplanar reconstructed T1-weighted rapid gradient echo sequence (MPRAGE, TE: 3.82 ms, TR 1900 ms, slice thickness: 1 mm, FOV: 256 mm), an axial spin echo proton density (PD)-weighted, a T2-weighted sequence (PD TE: 33 ms, T2 TE: 116 ms, TR 3540 ms, slice thickness: 5 mm, FOV: 230 mm) and a post-gadolinium T1-weighted sequence (TE: 10 ms, TR 500 ms, slice thickness: 5 mm, FOV: 230 mm).

MRI data analysis

All MRI scans were evaluated by two independent and experienced raters blinded for the clinical status of the patients. T2/PD-, T1 and T1Gd+ lesion volumes were measured with JIM Version 5.0 (Xinapse Systems, Ardwinckle, UK). Hyperintense lesions were marked on the PD scans and controlled by the T2 sequence. T2 lesions were marked when they appeared hyperintense in PD and T2. The rater had to exclude partial volume effects, perivascular spaces and other lesion-like hyperintensities. Confluent lesions were counted as one lesion. T1 black holes were defined as areas of signal hypointensity on contrast-enhanced T1 weighted images

with T2 hyperintense correlates. The intensity for black holes was defined as intermediate between cortical grey matter and CSF. T1 black hole volumes of the second scan were used to determine the black hole fraction ($BHFr = \text{hypointense lesion volume [T1vol]} \times 100 / \text{hyperintense lesion volume [PDvol]} - \text{volume of enhancing lesions [T1Gd+]}$) as a measure of focal tissue destruction.

Total and segmented intracranial volumes of grey matter, white matter and CSF of both scans were calculated using FAST v4.1, part of FSL 4.1.4 on MPAGE images [27,28]. In order to avoid detection of T1 hypointense lesions as grey matter, T1 lesion masks were implemented into the MPR sequence, using individually averaged normal appearing white matter (NAWM) values before brain tissue volume segmentation. Since FSL generates absolute grey and white matter volumes for each patient that make group comparisons difficult, we calculated fractions analogous to the standard brain parenchymal fraction (BPF) calculations. Whole brain parenchymal and segmented volumes were calculated as follows: $BPF [\%] = \text{grey matter volume (GMV)} + \text{white matter volume (WMV)} \times 100 / \text{total intracranial volume (TIV)}$, where $TIV = GMV + WMV + \text{CSF volume}$. Grey matter fraction ($GMF [\%] = GMV \times 100 / TIV$) and white matter fraction ($WMF [\%] = WMV \times 100 / TIV$) were calculated separately.

Spectral Domain Optical Coherence Tomography

Retinal measures of RNFL and total macular volume (TMV) were performed on a SD-OCT device (Heidelberg-Spectralis® SD-OCT, Heidelberg Engineering, Heidelberg, Germany, Spectralis software version 5.2.4.0, Eye Explorer software 1.6.2.0). This device incorporates a confocal scanning laser ophthalmoscope (CSLO) enabling it to perform active eye-tracking (TrueTrac™). Active eye-tracking is

used to adapt the software to eye movements and allows co-registration of the depth-scan with the CSLO fundus image. For RNFL, two 3.4 mm (12°) circular scans around the optic nerve head (ONH) consisting of 768 A-scans were acquired for each patient using the standard protocol by a qualified operator. The scan was performed in a darkened room and pupils were not pharmacologically dilated [30]. Quality of the OCT scans was rated by three independent readers. OCTs were excluded when all three raters rejected the scan for one or more of the following reasons: alignment- or algorithm failure, poor signal strength (<15), in cases, where the fundus was poorly illuminated, or if retinal pathology was detectable that would potentially impair RNFL readings. RNFL thickness (RNFLT) was calculated by the device's segmentation algorithm. For TMV, the built-in Fast Macular Volume protocol consisting of ... lines vertically crossing the macula was used.

Statistical Analysis

The study was a prospective observational study with a primary endpoint defined by a regression approach with BPF as target and RNFLT, history of optic neuritis and age as independent variables. We hypothesized a significant association of RNFLT with BPF when correcting for age, tested for by using Generalized Estimating Equations accounting for intra-patient inter-eye dependencies. Generalized Estimating Equations models (GEE) accounting for intra-patient inter-eye dependencies were used to assess the association between OCT and MRI using OCT parameters as independent- and MRI parameters as dependent variables and age as covariate. Additionally, solely for reasons of comparability with previously published studies, minimum RNFLT and minimum TMV were calculated as the lower respective value from each patient's eyes [17, 18]. E.g. if the right eye of a patient

showed a lower mean RNFL thickness than the left eye, the right eye's RNFL would have entered the analysis as this patient's minimum RNFL. Correlations between these minimum OCT values and MRI parameters were assessed using Spearman's Rho analysis. R^2 was calculated from linear regression models using MRI parameters as dependent- and OCT parameters as independent variables and correcting for age.

All statistical analyses were performed using IBM SPSS version 19 (IBM, Somers, NY, USA). A threshold of $p < 0.05$ was considered significant. All tests besides the primary endpoint should be understood as exploratory data analysis, in that no previous power calculation and adjustments for multiple testing were performed.

Sample size calculation

Sample size was calculated using G*Power 3.1.2 [30]. The sample size was calculated based on the results of the study by Gordon-Lipkin and colleagues [17]. Since no sample size calculation is available for GEE models applied here, sample size calculation was based on linear multiple regression models using the random model that supposes both target and predictor variables as random and should thus deliver a feasible estimation of the sample size required for GEE. A squared multiple correlation R^2 of 0.43 for RNFLT plus age and history of optic neuritis on BPF as previously reported [17] would be detected with 95% power ($\alpha=0.05$, two-sided, $k=3$ predictors) for $n=43$ patients.

Results

Patient and clinical characteristics

A total of forty-four patients were enrolled in this study to achieve a power of greater than 95%. The demographic data are summarized in table 1. Three RNFLT measurements were excluded by independent raters due to algorithm failure or poor signal strength. Nine TMV measurements were excluded from analysis due to poor signal strength or algorithm misalignment. Table 2 summarises the OCT data.

All MRI data sets could be analysed (table 2). The BHFr could be calculated from a total of 28 patients with a T2 lesion volume of >1ml. 22 eyes (25.9%) had a history of ON, 63 eyes (74.1%) did not. Minimum RNFLT correlated with age (Spearman's $Rho=-0.440$, $p=0.003$), and with minimum TMV (Spearman's $Rho=-0.363$, $p=0.017$) and BPF (Spearman's $Rho=0.453$, $p=0.002$). There were no correlations with the EDSS (minimum RNFLT $p=0.557$, minimum TMV $p=0.312$, BPF $p=0.079$) or disease duration (minimum RNFLT $p=0.327$, minimum TMV $p=0.749$, BPF $p=0.718$). The black hole fraction did not correlate with age ($p=0.880$) or EDSS ($p=0.238$). However, there was a correlation with disease duration ($Rho=0.401$, $p=0.035$) (all Spearman's Rho analyses).

Association between RNFLT, TMV and MRI

The results from GEE with MRI parameters as dependent variable, OCT parameters as independent variables and age and history of ON as covariate are given in table 3. The primary endpoint (RNFLT association with BPF with covariates age and history of ON) was significant ($p=0.005$). In an exploratory analysis using segmented volumes (WMF and GMF) as parameters for axonal and neuronal degeneration,

respectively, and the BHFr as parameter for focal tissue destruction, we observed an association between RNFLT and WMF as well as GMF. However, GEE analysis proved the association with GMF rather to be based on age-related effects, whereas the association between RNFLT and WMF appears to derive from true RNFLT effects (Table 3). Interestingly, there was no association between BHFr, reflecting focal atrophy or rather destructive focal lesions, and RNFLT in this model. Results from comparisons of TMV with MRI parameters were in line with those described for RNFLT (Table 3). GMV showed a similar association to age only, whereas the association from WMV with RNFLT was lost (not shown). There was no association between T2 lesion volume and black hole volume with either RNFLT or TMV (not shown). To rule out possible gender effects, we re-calculated each analysis, this time incorporating sex as a covariate, resulting in no change of significance levels (data not shown).

Minimum RNFLT and minimum TMV association with MRI

Next, we analyzed associations between OCT and MRI parameters using linear regression models with MRI parameters as dependent variables and OCT measures (minimum RNFLT; minimum TMV) as independent variables and age as covariate. Although these models do not take inter-eye dependencies into account, they should give an appropriate estimate of effect sizes and correlation in a diagnostic setting when interpreted carefully in combination with results from GEEs above. Furthermore, taking these data allows comparison of our results with data that had previously been published by others [17]. Results are summarised in table 3. Figure 1 shows results from linear regression on the association of minimum RNFLT with MRI parameters (BPF, GMF and WMF) not corrected for age. Taken together, the

linear regression models support the primary endpoint with an adjusted R^2 (minimum RNFLT and age) of 0.501 as well as – analogous to the GEE above - the associations between GMF and age, and between WMF and minimum RNFLT.

Discussion

Here we show that measures of retinal atrophy assessed by latest spectral domain OCT technology and age are inversely associated with brain MRI white- and grey matter damage in early forms of MS. Both RNFLT and TMV significantly explained the variance of WMF, whereas age did not. In contrast, age, but not RNFLT or TMV significantly predicted the variability in GMF. Our work confirms and extends the strong association of RNFLT and BPF, a measure of global brain atrophy, previously described by others [3, 17, 18, 20, 23, 31] in a highly homogeneous cohort of untreated MS patients with mostly early disease.

Moreover, our study assesses MRI/OCT associations with high-resolution spectral domain OCT and in a cohort of untreated CIS and early RRMS patients by a statistically pre-defined primary endpoint based on sample size calculations derived from findings of a previous report [17]. The most important observation of the present study is the independent association of OCT measures of retinal atrophy with MRI-derived WMF, but not GMF or BHFr when corrected for age and a history of optic neuritis, a finding that consistently resulted from both statistical models applied (GEE and linear regression models with minimum RNFLT and TMV). Our data – at least in part - contradict observations from previous publications, which yielded conflicting results [4, 17-20, 23]. A first study investigating the association between OCT measures and MRI brain atrophy in RRMS, secondary- and primary progressive MS

patients with higher EDSS and longer disease duration than our patients observed a significant correlation of minimum RNFLT, but not of TMV with BPF when correcting for age, and solely age-dependency of white matter volume (WMV) [17]. In contrast, grey matter volume (GMV) was neither associated with RNFLT nor age. A second study in a Spanish cohort by Sepulcre and colleagues – again with a heterogeneous cohort including CIS, RRMS, secondary- and primary progressive MS patients – reported significant correlations of average RNFLT with both WMV and GMV, whereas TMV was not investigated [18]. Siger et al. found a correlation of average RNFLT (mean from both eyes) only with GMF but not WMF in a subgroup of RRMS patients without a history of optic neuritis. However, the authors did not correct for age and examined a cohort with higher disease duration and EDSS when compared to the group of CIS and RRMS patients studied here [31]. Interestingly, no correlation between OCT and MRI measures was found by the same investigators in a subgroup of RRMS patients with a history of optic neuritis. Comparability between these results and ours is limited for several reasons: 1. different patient selection criteria with inclusion of heterogeneous disease courses, 2. heterogeneity with respect to disability and disease duration, 3. methodological differences in post-processing procedures for BPF and partial brain volume measurements, 4. application of time domain versus spectral domain OCT technology, 5. heterogeneous sample sizes, and 6. inhomogeneous retinal measures (mean versus minimum RNFLT) entering multiple regression analysis and statistical approaches. These differences may also explain the conflicting results regarding the association of OCT measures and GM atrophy, for which some authors have found a relationship [18, 20, 31] whereas others did not [17, 23].

Also, in our early MS cohort, we do not find a correlation of either OCT or MRI

atrophy measures with the EDSS which instead has been described in some but not all previous studies [16]. A possible explanation for this discrepancy might well be the overall very low EDSS scores in the patients described here with a median of 1.5 whereas higher EDSS scores are scarcely represented.

Our observation of a consistent association of both TMV and RNFL- assessed by the novel spectral domain OCT - with WMF but not GMF raises interesting questions regarding the underlying mechanisms and time course of MS-associated brain damage and injury to retinal axons, which are anatomically linked via the optic nerve. Thinning of the RNFL is considered to reflect damage to axons emerging from the retinal ganglion cells, thus CNS axons. Decrease of WMF may be the consequence of both demyelination and axonal loss, while loss of GM is believed to reflect damage to neuronal cell bodies. It is intriguing to hypothesize that in this early cohort with short disease duration in the majority of patients and low EDSS, OCT measures - irrespective of direct involvement of the optic nerve and the anterior visual pathway in MS pathology - partially also reflect more diffuse white matter damage remote from these sites. Further, we speculate that this white matter damage precedes later damage of neuronal cell bodies in the grey matter. In MS, degeneration detected by OCT measures of retinal atrophy might indirectly be associated with the extent of axonal damage in the brain compartment or be mediated by mechanisms like retrograde trans-synaptic degeneration, in case of lesions inflicting the posterior visual tract [6]. On the other hand, grey matter atrophy in the cohort reported here is predominantly driven by age and not associated with axonal degeneration depicted by OCT and WMF. Conceivably, a re-assessment of our patients at a later stage of their disease course might unravel an association of OCT measures also with grey matter damage, as with disease progression and increasing neuronal damage the

influence of the disease in relation to age on grey matter atrophy might prevail. Such a concept would suggest that, in early disease, autoimmune inflammation is mainly associated with demyelination, partial remyelination and to some extent axonal loss. When disease progresses secondary Wallerian degeneration and eventually loss of neuronal cell bodies may lead to disease- rather than age-related loss of grey matter. This might in part also explain the discrepancies with the data from other studies performed in patients that had advanced further into the disease and presented with a more progressive MS phenotype [17, 18].

A limitation of our study is the lack of a control group. However, we believe this highly unlikely to have a confounding effect on our data. Not only does one not expect significant thinning of the RNFL or volumetric measures in a group of healthy controls without ophthalmological comorbidity, nor did previous studies that have addressed this aspect by means of OCT and MRI in MS patients and controls [17, 18] find such loss or association in the respective control group.

RNFL thinning might only depict part of the overall retinal pathology in MS, and due to current methodological limitations, OCT measures might miss degeneration of layers below the level of retinal ganglion cell axons, like the ganglion cell- or inner and outer nuclear layers. Recent post-mortem and OCT studies suggest that a primary retinal pathology beyond damage to the RNFL may exist in a subgroup of MS or possibly also in the general population [32-34]. Our study suggests that, as soon as OCT segmentation algorithms become available to systematically identify MS phenotypes with an involvement of retinal cell bodies, MRI studies on grey matter pathology should be integrated.

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Authors' Contributions

KLY performed OCT measurements and wrote a first draft of the manuscript. AUB performed statistical analyses and revised the manuscript. AP revised the manuscript. LYR and FL performed the MRI measurements and post-processing of the scans. FP revised the manuscript. RM and SS conceived idea and protocol for this study, performed statistical analyses and drafted the final version of the manuscript.

Conflicts of interest

KLY, LYR and FL have nothing to disclose.

AUB is co-founder, shareholder and director of gfnmediber GmbH, received speaker's honoraria from Heidelberg Engineering, travel grants from Biogen Idec and has research collaborations with Heidelberg Engineering and Carl Zeiss Meditec.

AP has served (2009) on one single scientific advisory board meeting for and has received funding for one single travel from Novartis; serves on the editorial board of *Multiple Sclerosis International*; receives royalties from the publication of "Die Anaemien" (Engl anaemias) (Shaker Verlag, 1999); served on the advisory board for the (EA)YNT-Schering fellowship for clinical training in multiple sclerosis (2000-2005) and has received research support from the Dutch MS research Foundation.

FP has research collaborations with Heidelberg Engineering and Carl Zeiss Meditec. RM has received compensation for advisory board activities or for giving lectures at workshops/clinical meetings by Teva, Sanofi-Aventis, Biogen Idec, Merck & Serono. He further received support by Biogen Idec, Merck & Serono and Novartis for unrestricted project grants.

SS together with FP sits on the Novartis steering committee for a multicentre, longitudinal OCT study, and receives honoraria. He further received unrestricted research grants from Biogen Idec and Bayer Schering and consulting and speaker's fees from Bayer Schering, Biogen Idec, Merck-Serono, Novartis and sanofi-aventis.

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Figure Legends

Figure 1

Association of RNFLT with BPF (A) and WMF (B). Solid lines are derived from a linear regression analysis with minimum RNFLT as independent and the respective MRI parameter as dependent variable. R^2 from these linear models are given in the upper right corner of each figure section. Dashed lines represent 95% confidence intervals. **Abbreviations:** RNFLT = retinal nerve fibre layer thickness, BPF[%] = brain parenchymal fraction, WMF[%] = white matter fraction.

Table 1. Patient demographics and clinical data

Subjects	<i>n</i>	44
Gender	Male (%)	9 (20.5%)
	Female (%)	35 (79.5%)
Age [years]	Mean (SD)	41.4 (8.8)
	Min - Max	24 - 60
Diagnosis	RRMS (%)	34 (77.3%)
	CIS (%)	10 (22.7%)
Disease duration [years]	Mean (SD)	3.2 (2.7)
	Min - Max	0.1 - 8.9
EDSS	Median	1.5
	Min - Max	0.0 - 4.5
Eyes	<i>n</i>	85
History of Optic Neuritis	No (%)	63 (74.1%)
	Yes (%)	22 (25.9%)
Visual Acuity (high contrast)	Mean (SD)	1.03 (0.14)
	Min - Max	0.66 - 1.25

EDSS= Expanded Disability Status Scale; RRMS=Relapsing remitting multiple sclerosis;
CIS=Clinically isolated syndrome

Table 2. OCT and MRI data

	Mean	SD	Min	Max
OCT				
RNFLT Average [μm]	92,5	10,2	63	112
RNFLT Temporal Quadrant [μm]	67,5	15,2	33	111
RNFLT Superior Quadrant [μm]	108,1	14,5	74	145
RNFLT Inferior Quadrant [μm]	121,6	16,3	80	162
RNFLT Nasal Quadrant [μm]	72,1	13,3	47	117
Macular Volume TMV [mm^3]	8,34	0,39	7,58	9,14
MRI				
T1 black hole volume (BHV) [ml]	0,51	1,44	0	8,2
T2 lesion volume (T2V) [ml]	2,55	3,18	0,06	15,42
T1 Gd enhancing lesion volume (GEV) [ml]	0,07	0,14	0	0,62
Black hole fraction (BHFr=BHV/T2V)	13%	16%	0%	55%
CSF [ml]	399.899	56.171	301.433	569.291
GMV [ml]	839.906	76.720	693.391	1.011.498
WMV [ml]	577.893	64.175	483.454	699.297
BPF ((GMV+WMV)/(GMV+WMV+CSF))	78,0%	2,1%	73,7%	82,3%
GMF ((GMV)/(GMV+WMV+CSF))	46,0%	2,0%	43,0%	49,0%
WMF ((WMV)/(GMV+WMV+CSF))	32,0%	1,0%	29,0%	35,0%

RNFLT=Retinal nerve fibre layer thickness; PMB=Papillomacular bundle; TMV=Total macular volume; BHFr=Black hole fraction; CSF=Cerebrospinal fluid; GMV=Grey matter volume; WMV=White matter volume; BPF=Brain parenchymal fraction; GMF=Grey matter fraction; WMF=White matter fraction

Table 3. GEE and linear regression models for predicting MRI measures from OCT data

	BPF	GMF	WMF	BHFr
LR Adjusted R² for age + minimum RNFLT	0.501	0.353	0.395	0.114
GEE p-value for age	<0.001	<0.001	0.429	0.818
LR R ² change for age (p-value)	0.265 (<0.001)	0.374 (<0.001)	0.012 (0.499)	0.003 (0.798)
GEE p-value for RNFLT	0.005	0.717	0.003	0.994
LR R ² change for minimum RNFLT (p-value)	0.237 (<0.001)	0.026 (0.211)	0.319 (<0.001)	0.012 (0.621)
LR Adjusted R² for age + minimum TMV	0.374	0.334	0.220	0.066
GEE p-value for age	<0.001	<0.001	0.442	0.578
LR R ² change for age (p-value)	0.260 (<0.001)	0.366 (<0.001)	0.012 (0.502)	0.017 (0.547)
GEE p-value for TMV	0.034	0.357	0.032	0.616
LR R ² change for minimum TMV (p-value)	0.127 (0.008)	0.017 (0.322)	0.158 (0.008)	0.039 (0.372)

GEE=Generalized Estimating Equation Models; BPF=Brain parenchymal fraction; GMF=Grey matter fraction; WMF=White matter fraction; BHFr=Black hole fraction; RNFLT=Retinal nerve fibre layer thickness; TMV=Total macular volume

